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Research Progress and Forecast Report
(9/30/84 - 4/30/85)

PHOSPHOPROTEIN REGULATION OF SYNAPTIC REACTIVITY: ENHANCEMENT AND CONTROL OF A MOLECULAR GATING MECHANISM

Forecast Report for AFOSR 83-0335
Submitted to:
Directorate of Life Sciences
Air Force Office of Scientific Research

Submitted by:
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I. Significant Accomplishments and Research Progress

(1) Fatty acid regulators of protein kinase C.

We have demonstrated that the regulation of synaptic reactivity is closely associated with phosphorylation of protein F1 and that this phosphorylation depends on the activation of protein kinase C.

As recently described in the Annual Progress Report we have achieved protein kinase C purification to near homogeneity. This accomplishment paves the way for evaluating unambiguously kinase C regulation. In a system that contains only kinase, substrate and essential co-factors, the regulation of the C kinase byphosphatidylserine (PS) has been confirmed. In the presence of PS, diolein increases the affinity of kinase C for calcium. Thus, the Ka for Ca++ is 30 uM in the absence of diolein and only 1.3 uM in its presence.

We have examined the role of the fatty acid chains released from the PS molecule by phospholipase A2 and phosphnolipase C and have found that oleic acid and arachidonic acid activate protein kinase C while diolein and the methyl esters of oleate and arachidonate do not activate C kinase. The polar head group or related compounds had no influence on the stimulation of protein kinase C.

In the next six months we plan to evaluate further the relative potency of saturated and unsaturated fatty acids as

independent regulators of protein kinase C. In addition, we shall begin to evaluate the direct effects of peptide hormones on kinase C activity using the purified enzyme preparation. Finally, we shall study receptor mediated effects of these peptides (ACTH, opioids, insulin) using preparations known to stimulate the phosphorylation of protein F1 (Akers & Routtenberg, 1984).

We plan to interface these results with electrophysiological studies aimed at enhancing synaptic reactivity with micro-iontophoresis of kinase C stimulating agents. These experiments will be described in the last section of the forecast report.

(2) Endogenous protein inhibitor of protein kinase C,

As briefly mentioned in the Progress Report we have discovered an endogenous inhibitor of protein kinase C. The discovery was made during the initial purification steps for protein F1. It was noticed that kinase C was unable to phosphorylate the acid extract containing protein F1. This puzzling result was clarified by the finding that ammmonium sulfate precipitation of the extract caused the kinase to phosphorylate protein F1. It was then reasoned that this step may have removed an inhibitor of protein kinase C. This inhibitor has been shown to be a protein by inactivation experiments using heat or trypsinization. This has lead to a

protein purification regimen using the hydroxyapatite column. We have now obtained a purified inhibitor fraction from the column that completely inhibits protein kinase C activity. It will be of importance to determine its subcellular location as one would expect that there would be co-localization of the C kinase and its physiological inhibitor.

These findings will be useful in our micro-iontophoresis studies aimed at regulating synaptic reactivity by ejection of compounds known to regulate protein kinase C activity.

(3) Exogenous micro-iontophoretic application of protein kinase C regulators.

These experiments have just recently been initiated. In this report I shall describe the techniques used and the rationale for the protocols instituted.

Five-barreled micropipettes are placed within the hilar zone of the dentate gyrus. Our initial experiments are directed at determining both the electrophysiological and biochemical consequences of our ejections. Therefore, we have chosen an ejection site that will access a reasonable extent of the dentate gyrus in the dorsal hippocampus. Drug distribution will be evaluated using tritiated forms of the ejected compound followed by dissecting tissue at known distances from the ejection site and counting tritium levels.

The following protocol is now being used. Stimulating

electrodes are placed within the bottleneck of Lomo to maximally activate the dorsal hippocampal dentate gyrus. Initial exploration is performed with a single barreled micro-pipette. Then the multibarreled pipette is lowered into the hilar location indicated by the initial exploration. Backing currents are applied to all ejecting barrels. Characterization of baseline electro- physiological parameters (Input/output; paired pulse; laminar profile) is then followed by a 10 min ejection at typically less than 100 nA. The attempt is to access the molecular layer of both the dorsal and ventral leaf of the dentate gyrus. Following the ejection the effects are then evaluated on baseline parameters. To date agents that influence kinase C do not have any significant influence on these parameters. This is consistent with our finding that protein kinase C does not appear to be altered by synaptic activation in the absence of a change in synaptic reactivity.

We then allow the parameters to return to baseline before assessing the effects of ejection of both a control vehicle and then a kinase C regulator on long-term potentiation parameters. The rationale for this approach is that kinase C stimulation alone may not be sufficient to bring the enzyme in proximity to protein F1. For example, an elevated intracellular calcium level is likely to be necessary in translocation of the kinase to and intercalation with the synaptic membrane.

Titration of the high frequency parameters is clearly necessary since too intense stimulation could fully activate the kinase C system, allowing little room for the action of exogenous compounds. We are currently exploring this parameter using a constant pulse train and varying the number of trains. In this way we plan to maximize the effects of kinase C manipulation.

At this juncture we have decided on a within-animal design to minimize inter-animal variation. In the overall protocol, then, control and experimental ejections will be counterbalanced so that the cumulative effects of high frequency stimulation are not a contributing factor.

Reagents isolated in the biochemical laboratory can now be studied with micro-iontophoretic methods. We plan, for example, to evaluate the electrophysiological consequences of phospholipids, fatty acids and protein kinase inhibitors in the hippocampal formation.

Finally, at the completion of the experiment we shall determine the effects of the reagents ejected in vivo on phosphorylation and protein kinase C activity measured in vitro. By measuring the biochemical consequences of the iontophoretic ejection it may be possible to establish a direct relation between kinase C stimulation and the enhancement of synaptic reactivity.

(4) Acquisition and Construction of Major Research Equipment.

On December 15, 1984 a VAX 11/750 was installed in this laboratory. The funds for the equipment were provided by the University Research Instrumentation Program of DoD (AFOSR 84-0260). Considerable time and effort was directed at site preparation and realignment of other laboratory functions. There is now an ongoing process of integrating the computer within the laboratory environment, as laboratory personnel become familiar with its many utilities and programmers provide user friendly interfaces to upload electrophysiological and biochemical data from the micro- to the mainframe environment.